ISSN 1070-3632, Russian Journal of General Chemistry, 2014, Vol. 84, No. 7, pp. 1411–1416. © Pleiades Publishing, Ltd., 2014.

Synthesis, Spectroscopic, and Molar Conductance Characterization of Selenium(IV) Vitamin B6 Complex as Prospective Antioxidant Agent[1]

Moamen S. Refat[a,b], Mohamed I. Kobeasy[a,c], and Manal A. EL-Shazly[d,e]

[a] *Chemistry Department, Faculty of Science, Taif University, Al-Haweiah, P.O. Box 888, Zip Code 21974, Taif, Saudi Arabia*
e-mail: msrefat@yahoo.com

[b] *Chemistry Departments, Faculty of Science, Port Said University, Egypt*

[c] *Faculty of Agriculture, Biochemistry Department, Cairo University, Giza, Egypt*

[d] *Faculty of Science, Biology Department, Taif University, Saudi Arabia*

[e] *Virus Research Department, Plant Pathology Research Institute, Agriculture Research Center (ARC), Giza 12619, Egypt*

Received February 25, 2014

Abstract—Selenium(IV) complex of vitamin B_6 has been prepared using Se(IV) tetrachloride and characterized using spectroscopy (IR, UV-Vis, H NMR), molar conductance measurements, thermal analysis (DTA and TGA) and SEM imaging. Micro-analytical and spectral data show that the formed selenium(IV) complex is 1 : 2 (Se : vitamin B_6) molar ratio. Vitamin B_6 and its selenium complex were screened for in vitro antioxidant activity. The complex exhibited stronger antioxidant activity in 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical assay compared to the free vitamin B_6 ligand.

Keywords: vitamin B_6, spectroscopic, thermal, antioxidant assessment

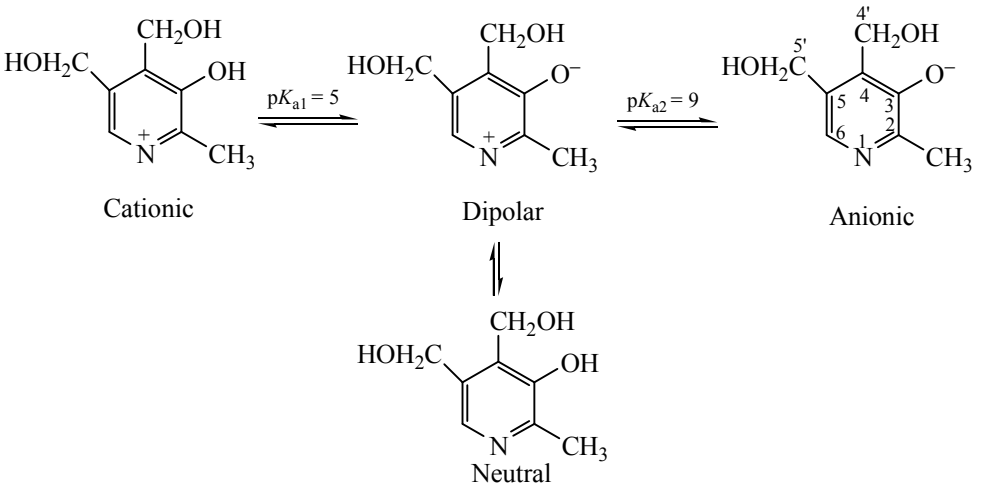
DOI: 10.1134/S1070363214070299

INTRODUCTION

Pyridoxine [2-methyl-3-hydroxy-4,5-bis(hydroxymethyl)pyridine] and its synonyms are adermine and vitamin B_6 (Fig. 1). A series of vitamin B_6 pyridoxol complexes with La, Ce, Pr, Nd, Sm, Eu, Gd, Ho, Er(III), and Y, $M(PN)_5Cl_3{\cdot}6H_2O$ (where PN = pyridoxol), were synthesized and antioxidative activity of complexes was determined [1]. Pyridoxine under various pH conditions exhibits four interchangeable ionic forms, i.e. at pH < 5 occurs as cationic, neutral form and dipolar ion at pH 6.8, and anionic at pH > 8 [2]. Scheme 1 shows the equilibria and the referenced values of dissociation constants (pK_a) between these species in aqueous solution. Ultraviolet spectra and acid dissociation constants, [3] as well as nuclear magnetic resonance studies [4] of pyridoxine and pyridoxamine show that the loss of the first proton corresponds to the 3-hydroxy proton. The second ionization results in the loss of the pyridinium proton. The crystal structure of pyridoxine hydrochloride shows that both the pyridine nitrogen and the phenolic oxygen were protonated, as expected [5].

Pyridoxine displays different coordination sites with metal ions with various charges and "hard"/"soft" character. Chelation through phenolate oxygen and adjacent hydroxymethyl groups is common for pyridoxine metal complexes [6, 7]. Other bonding modes of pyridoxine are (1) simple coordination through the pyridine nitrogen [6]; (2) chelation plus bonding through the pyridine nitrogen [8]; and (3) chelation plus bridging through the coordinated phenolate or hydroxyl group [9]. Vitamin B_6 is an essential water soluble vitamin required for normal growth and development and it functions as a cofactor in numerous enzymic reaction of amino acids, carbohydrates, neurotransmitters and lipid metabolism [10]. The immune and nervous systems need vitamin B_6 to function efficiently and it's also necessary for red blood cell metabolism [11]. Pyridoxine seems to quench singlet oxygen at a rate comparable with that

[1] The text was submitted by the authors in English.



Scheme 1. Dissociation equilibria of vitamin B_6 in aqueous solution.

of vitamins C and E. Thus vitamin B_6 seems to be involved in active oxygen resistance and has antioxidant activity which suppress the progression of homocysteine-induced atherosclerosis [12]. Vitamin B_6 decreases the level of homocysteine by *trans*-sulfation to cysteine thus low levels of vitamin B_6 may cause hyperhomocysteinemia and increase the risk for atherosclerosis [13].

Organo-selenium and organo-tellurium compounds have pharmacological properties as antioxidant, immunomodulating and anti-inflammatory agents [14]. Selenium is an essential component of glutathione peroxidase (GP_X), which play a critical role in protecting aerobic organisms from oxygen radical–mediated injuries [15] and selenium deficiency accompanied with decreasing in GP_X activity also lows concentration of selenium compounds that protect the tissues against lipid peroxidation and have the ability to reduce the thiobarbituric acid reactive substances (TBARS) production to the basal level [15]. Therefore, the synthesis of new organoselenium compounds with antioxidant properties is highly desirable for the development of potential therapeutic agents especially those that can mimic physiological activities [16]. Literature survey reveals no recent records on the complexation between selenium(IV) with vitamin B_6. It was a thought of interest to study the synthesis and characterization, thermal behavior and antioxidant screening of the Se(IV) complex of the vitamin B_6 ligand.

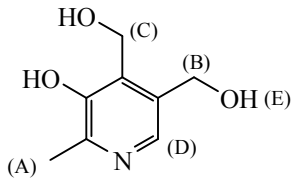


Fig. 1. Structure of vitamin B_6.

EXPERIMENTAL

All chemicals, solvents, pyridoxine hydrochloride, selenium(IV) chloride were commercially available from Aldrich chemical company and were used without further purification.

Elemental analysis have been carried out using Vario EL CHNS instrument. The molar conductance of 10^{-3} M solutions in DMF were measured on a Jenway 4010 conductivity meter. IR spectra were recorded on Bruker infrared spectrophotometer in KBr pellets in 400–4000 cm^{-1} range. The electronic spectra were measured in 1.0×10^{-3} M DMSO solution in 200–900 nm range using Unicam UV-Vis spectrometer. The proton NMR spectra were recorded on a Varian FT-300 MHz spectrometer in DMSO-d_6, using TMS as internal standard. SEM images were obtained using a Jeol Jem-1200 EX II Electron Microscope at an acceleration voltage of 25 kV. X-ray diffraction (XRD) patterns of the samples were recorded on an XPert Philips X-ray diffractometer. All the diffraction patterns were obtained by using Cu$K_{\alpha 1}$ radiation, with a graphite monochromator at 0.02°/min scanning rate. Differential Thermal Analysis (DTA) and Thermogravimetric Analysis (TGA) experiments were conducted using Shimadzu DTA-50 and Shimadzu TGA-50H thermal analyzers under nitrogen atmosphere at a flow rate of 30 mL/min and a 10°C/min heating rate for the 25–800°C temperature range.

Antioxidant assessments. DPPH radical scavenging activity: The antioxidant activity of vitamin B_6 and its selenium(IV) complex were determined based on the radical scavenging ability in reacting with stable DPPH free radical according to [17]. The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation RSA, % = $[(A_{DPPH} - A_S)/(A_{DPPH})] \times 100$ were AS is the absorbance of the solution when the sample has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. Ascorbic acid was used as standard. All tests and analyses were done in triplicate and the results were averaged.

Preparation of selenium(IV) vitamin B_6 complex. 2 mmol of pyridoxine hydrochloride was dissolved in 25 mL of methanol then mixed with 25 mL of methanolic solution of 1 mmol of selenium(IV) tetrachloride. pH of the mixture was adjusted to 8–9 by adding 1 M methanolic ammonia solution and the whole was heated under reflux and continuous stirring at 60–70°C for about 2 h. The mixture was left overnight until precipitation settled down. The precipitate resulted was filtered off and washed with methanol then left over anhydrous calcium chloride in desiccator. Yield 75%, brown powder, soluble in hot dimethylsulfoxide and dimethylformamide but insoluble in water and some other organic solvents, mp 178°C. Calculated, %: C 34.21; H 3.88; N 4.95; Cl 25.11; Se 13.96. $C_{16}H_{22}Cl_4N_2O_6Se$. Found, %: C 34.37; H 3.97; N 5.01; Cl 25.36; Se 14.12. *M* 559.13.

RESULTS AND DISCUSSION

The elemental analysis of synthetic Se(IV) pyridoxine complex is 1 : 2 [Se(IV): vitamin B_6] molar ratio. The molar conductance values for 10^{-3} mol/cm^3 concentration of Se(IV) complex is 101 Ω^{-1} cm^2 mol^{-1}. However, the low value of 16 Ω^{-1} cm^2 mol^{-1} was recorded for the free vitamin B_6 ligand. This suggests that Se(IV) complex is of electrolytic nature [18, 19]. The high value of the molar conductance of this complex may be due to the sharing of the chloride ions in the chelation along with the hydrochloride pyridoxine. The presence of two hydrochloric molecules in the complex was detected with a few drops of the concentrated silver nitrate reagent and the appearance of the white precipitate.

The infrared absorption bands are one of the important tools of analyses used for determining the mode of chelation. Pyridoxine behaves as an ionic bidentate molecule, coordinating to the metal ions through the C^4–CH_2OH and the deprotonated phenolic groups. Comparing with the published data [20], the assignments of bonding sites are summarized as follows. The spectrum of Se(IV) complex shows a broad band in the region 3500–3000 cm^{-1}, which can be attributed to ν(OH) and ν(C–H) vibration motions of the C^5–CH_2OH group, ν(C–H) of the pyridine ring and ν(C–H) of methyl group, respectively [21]. The range 1634–1433 cm^{-1} proves the pyridine ring bands, and the expected [22] strong IR band at about 1530 cm^{-1}; probably for the ν(C–O) of the coordinated phenolate group. The strong bands close to 1000 cm^{-1} are assigned [23] to ν(C–O) of the C^5–CH_2OH group. The Se(IV) complex has a *trans*-configuration as indicated by the observation of only one band for the M–O vibration at ~580 cm^{-1} [24].

The electronic spectra of the free vitamin B_6 ligand and its Se(IV) complex were recorded in DMSO, and the data obtained correspond to the π→π* and *n*→π* transitions. The first range can be assigned to π→π* transitions in the aromaticity of the pyridine ring, while the second range is most probably due to the *n*→π* transitions of OH groups and of the nitrogen atom of the pyridine ring [25]. The third type of transition in the visible region at >400 nm for this complex can be attributed to the ligand-to-metal charge transfer bands LMCT; due to the transfer of the electronic lone pairs of the hydroxyl group oxygen to the metal ions. The pyridoxine hydrochloride free ligand possesses absorption at 272, 288, 300, 314, 338, and 375 nm regions. The absorption bands of the Se(IV) complex were observed at 280, 286 nm [25] belonging to π→π* transitions and at 328, 332, 340, 364, and 378 nm, respectively, due to the *n*→π* transitions of OH groups and of the nitrogen atom of pyridine ring [25]. The third type of the transition in visible region above 400 nm can be attributed to the ligand-to-metal charge transfer bands LMCT; i. e. the electronic lone pairs of the oxygen in the hydroxyl group transfer to the metal ions, but it is hardly noticed for these complexes due to the broadness of the spectra.

^{1}H NMR spectra of pyridoxine HCl free ligand and its selenium(IV) complex were recorded in DMSO-d_6. Pyridoxine HCl free ligand has the following peaks δ, ppm: 2.34 [$3H^{(A)}$, CH_3 group], 4.50 [$2H^{(B)}$, CH_2 group], 4.76 [$2H^{(C)}$, CH_2 group], 8.17 [$1H^{(D)}$, CH group] and 5.21 [$1H^{(E)}$, OH group] [26]. The Se(IV) complex shows the following peaks δ, ppm: 2.38 [$3H^{(A)}$,

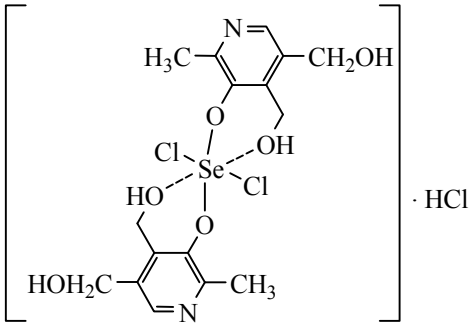


Fig. 2. Suggested structure of Se(IV) pyridoxine HCl complex.

CH_3 group], 4.54 [$2H^{(B)}$, CH_2 group], 4.73 [$2H^{(C)}$, CH_2 group], 7.92 [$1H^{(D)}$, CH group] and 5.73 [$1H^{(E)}$, OH group]. These results can explain that in the case of Se(IV) complex, the signals of A, B, C, and D hydrogens, were blue shifted in comparison with the free pyridoxine HCl ligand, where it can be ascribed to the participation in the chelating process. On the other hand, the decrease in the intensities of hydroxyl group confirms that, this hydroxyl group is participating in the coordination with the central metal atom without proton displacement. On the basis of the above studies, the suggested structure of the pyridoxine selenium(IV) complex can be presented as shown in Fig. 2.

SEM imaging. The microstructure, surface morphology and chemical composition of the free pyridoxine ligand and its selenium(IV) complex were studied using scanning electron microscopy. SEM micrograph reveals the well sintered nature of the complex with variant grain sizes and shapes. The uniformity and similarity between the particles size, shape and forms of the synthesized pyridoxine complex indicate that morphological phases of Se(IV) complex has inhomogeneous matrix. Clear large grains are obtained with agglomerates for pyridoxine free ligand. Un-homogeneous phase formation of selenium(IV)pyridoxine complex has small-to-medium particle size of different shape. The particle size distribution of this complex is evaluated and the average particle size is found to be 20 μm. It can be concluded that the significance of this result is in the conversion of agglomerate particles of free vitamin B_6 to nano particles upon the complexation with Se(IV) ions.

X-ray studies. The X-ray powder diffraction studies in the range of $10° < 2\theta < 80°$ for the free vitamin B_6 ligand and its Se(IV) complex were carried out in order to obtain an idea about the lattice dynamics of the resulted complex. The maximum diffraction patterns of vitamin B_6 and its Se(IV) complex exhibit at $2\theta/d$-value (Å) = 20.73/4.281 and 28.41/3.210, respectively. The crystallite size could be estimated from XRD patterns by applying FWHM of the characteristic peaks using Deby-Scherrer equation [28].

$$D = K\lambda/\beta\cos\theta, \quad (1)$$

where D is the particle size of the crystal gain, K is a constant (0.94 for Cu grid), λ is the X-ray wavelength (1.5406 Å), θ is the Bragg diffraction angle and β is the integral peak width. The particle size is estimated according to the highest value of intensity compared with the other peaks. The X-ray obtained for selenium(IV) complex give an impression that the particle size is located within the nano scale range.

Thermal analythes. The TG curve of the pyridoxine hydrochloride free ligand shows two main consecutive steps of mass loss at the temperature range 40–662°C. At the first step at 40–390°C, the mass loss of 41.11% with maximum rate (T_{DTG}) at 212.6°C, corresponds to an endothermic volatilization of hydrogen chloride molecule together with $CH_2(OH)_2$ fragment (calculated = 41.10%) at T_{DTA} of 204.8°C. The mass loss (58.89%) at the second step (390–662°C) of T_{DTG} at 464.7°C and at 575°C is assigned to the exothermic release of C_7H_7NO fragment (calcd. = 58.90%) at T_{DTA} of 464.5°C. Although the T_{DTG} of this second step shows two overlapping peaks, the TG curve appears as a continuous mass loss with no plateau.

The TG curve of $[Se(VitB_6)_2(Cl)_2]\cdot 2HCl$ complex shows two main consecutive steps of mass loss at the temperature ranges 180–400°C and 400–600°C. At the first step 180–400°C, the mass loss of 80.00% with maximum rate (T_{DTG}) at 275°C, corresponds to an endothermic volatilization of two pyridoxine HCl molecules together with chlorine (Cl_2) gas moiety fragment (calculated = 80.16%) at T_{DTA} of 225 °C. The mass loss (20.00%) at the second step (400–600°C) of T_{DTG} at 500°C as assigned to the exothermic complete release of selenium dioxide (sublimated) fragment (calculated = 19.84%) at T_{DTA} of 510°C.

In vitro antioxidant assessment. Antioxidant activity of vitamin B_6 and vitamin B_6 selenium complex were carried out by DPPH radical scavenging assay. This assay is considered to be good in vitro model widely used to assess free radical scavenging efficacy in a relatively short time and could be taken as

Antioxidant activity (%) of vitamin B_6 and vitamin B_6 selenium complex[a]

Compound	Concentration, μg/mL					
	5	10	25	50	100	200
Vitamin B_6	9	15	19	25	32	41
Vitamin B_6 selenium complex	13	18	23	28	37	52
Ascorbic acid (standard)	17	25	30	43	53	75

[a] Against DPPH radical scavenging assay.

an indication of the hydrogen donating ability of the tested compounds. This assay was selected to its simplicity and worldwide acceptance and enabled us to compare results [29]. DPPH in free radical from has an absorbance at 515 nm which disappears upon reduction by an antioxidant compound to become a stable diamagnetic molecule with a result of a color change from purple to yellow [29]. Hence, DPPH is usually used as substrate to evaluate antioxidative activity of antioxidants [30]. Results on inhibition percentage of DPPH radical by tested compounds are presented in the table.

The scavenging effects of vitamin B_6 and vitamin B_6 selenium complex on DPPH radical increased with concentration, vitamin B_6 selenium complex has shown stronger DPPH scavenging activity than vitamin B_6. These results indicated that antioxidant activity of vitamin B_6 may be due to hydrogen atom donation as the main mechanism of radical-scavenging activity of vitamin B_6 and vitamin B_6 may contribute to the total antioxidant capacity of antioxidants present in human body especially at basic conditions [11], also antioxidant activity of vitamin B_6 is in decreasing plasma lipid peroxidation and an increased tissue superoxide dismutase activity [31]. It is suggested that vitamin B_6 penetrates cell membrane easily and is taken into the red blood cells and phosphorylated by pyridoxal kinases found in erythrocytes [32]. The coenzyme form of vitamin B_6 is remarkably versatile, being involved in transaminations, decarboxylations, racemizations and numerous modifications of amino acid side chains. Among these reactions pyridoxal-phosphate is implicated in the metabolism pathway for the formation of cysteine, which is the rate limiting forerunner in glutathione synthesis and reduced glutathione is a major antioxidant within and without the cell and is also responsible for maintaining the redox status of the cell through the GSH/GSSG ratio [33]. Also deficiency in vitamin B_6 causes impairment in the antioxidant defense system and leads to excessive free radical production in rats liver tissue thus vitamin B_6 seems to be associated in some defense mechanism especially against lipid peroxidation in tissue [14]. On the other hand the high antioxidant activity of vitamin B_6 selenium complex may be caused by protective role of selenium compounds through multiple antioxidant mechanisms such as (1) selenium compounds have ability to scavenge reactive oxygen species (ROS) such as peroxyl radical, superoxide radical and peroxynitrite radical which mediated DNA damage [34]. (2) Selenium compounds also exhibit antioxidant activity by increasing the activity of glutathione peroxidase which reduces peroxides radicals by the GP_X catalytic cycle which produce selenonic acid (PSeOH) reacting with glutathione (GSH) to generate a selenenyl-sulfide adduct (PSeSG). The adduct reacts with an additional GSH to generate the active selenol (PSeH) that reduces peroxide [35]. (3) Organic selenium compounds express antioxidant activity by metal binding to prevent copper mediated DNA damage through making Cu–Se coordination and show positive shifts in the copper reduction potential and protect cell components against metal toxicity and oxidative stress.

REFERENCES

1. Yang, K.W., Wang, L.F., Wu, J., and Yang, Z.Y., *J. Inorg. Biochem*., 1993, vol. 52, p. 151.
2. Metzler, D.E., and Snell, E.E., *J. Am. Chem. Soc*., 1955, vol. 77, p. 2431.
3. Matushima, Y., and Martell, A.E., *J. Am. Chem. Soc*., 1967, vol. 89, p. 1322.
4. Lapper, R.D., Manisch, H.H., and Smith, I.C.P., *Can. J. Chem*., 1975, vol. 53, p. 2406.
5. Hanic, F., *Acta Crystallogr*., 1966, vol. 21, p. 332.
6. Back, D.F., de-Oliveira, G.M., and Lang, E.S., *J. Inorg. Biochem*., 2006, vol. 100, p. 1698.
7. Neelakantan, M.A., Sundaram, M., Thalamuthu, S., and Nair, M.S., *J. Coord. Chem*., 2010, vol. 63, p. 1969.

8. Chamayou, A.C., Neelakantan, M.A., Thalamuthu, S., and Janiak, C., *Inorg. Chim. Acta*, 2011, vol. 365, p. 447.
9. Acquaye, J.H.K.A. and Richardson, M.F., *Inorg. Chim. Acta*, 1992, vol. 201, p. 101.
10. Denslow, S.A., Walls, A.A., and M.E. Daub, *Physiological and Molecular Plant Pathology*, 2005, vol. 66, p. 244.
11. Gliszczynska-swiglo, A., and Szmusiak, H., *Pol. J. Food Nutr. Sci*., 2007, vol. 57, p. 163.
12. Endo, N., Nishiyama, K., Otsuka, A., Kanouchi, H., Taga, M., and Oka, T., *British J. Nutrition*, 2006, vol. 95, p. 1088.
13. Choi, E., and Cho, Y., *Nutrition Research and Practice*, 2009, vol. 3, p. 208.
14. Sabir, S.M., Salman, S.M., Rocha, J.B.T., *Enviromental Toxicology and Pharmacology*, 2012, vol. 43, p. 446.
15. Bordoni, A., Cabrini, L., Marchetti, M., Danesi, F., Bochicchio, D., Biagi, P.L., and Maranesi, M., *Ann. Nutr. Metab*., 2006, vol. 50, p. 305.
16. Vivane, P.S. Danubia, B.S., Marcio, W.F., Nogueira, C.W., Rocha, J.B.T., and Gilso, Z., *Chem. Biol. Inter*., 2009, vol. 177, p. 28.
17. Biois, M.S., *Nature*, 2002, vol. 26, p. 1199.
18. Geary, W.J., *Coord. Chem. Rev*., 1971, vol. 7, p. 81.
19. Refat, M.S., *Spectrochim Acta A*, 2007, vol. 68, p. 1393.
20. Acquaye, H.K.A., *Master Thesis*, Brock University, St Catherine's, Ontario, Canada, 1982.
21. Tabassum, S., Siddiqi, K.S., Khan, N.H., Kureshy, R.I., and Zaid, S.A.A., *Indian J. Chem*., 1987, vol. 26, p. 523.
22. Nakamoto, K., *Infrared Spectra of Inorganic and Coordination Compounds*, 2nd ed., New York: Wiley-Interscience, 1970.
23. Agarwal, R.K., Garg, R.K., and Sindhu, S.K., *J. Iranian Chem. Soc*., 2005, vol. 2(3), p. 203.
24. Franklin, T.A., and Richardson, M.F., *Inorg. Chim. Acta*, 1980, vol. 46, p. 191.
25. Lever, A.B.P., Mantiovani, E., and Ramaswamy, B.S., *Can. J. Chem*., 1971, vol. 49, p. 1957.
26. Singh, H.L., and Varshney, A.K., *Bioinorg. Chem. Appl*., 2006, doi:10.1155/BCA/2006/23245.
27. Radhakrishnan, P.S., and Indrasenan, P., *Indian J. Chem*., 1989, vol. 28, p. 234.
28. Cullity, B.D., *Elements of X-ray Diffraction*, Addison-Wesley, Mass, 1978.
29. Nantitanon, W., and Okonogi, S., *Durg Discoveries and Therapeutics*, 2012, vol. 6, p. 38.
30. Shimada, K., Fujikama, K., Yahara, K., and Nakamura, T., *J. Agricultural and Food Chemistry*, 1992, vol. 40, p. 945.
31. Pahlavanzadeh, F., Bidadkosh, A., Derakhshanfar, A., Rastegar, A.M., and Rushanzamir, M., *Comparative Clinical Pathology*, 2013, vol. 22, p. 637.
32. Stocker, P. Lesgards, J., Vidal, N., Chalier, F., and Prost, M., *Biochimica et Biophysica Acta*, 2003, vol. 1621, p. 1.
33. Schafer, F.O., and Buettner, G.R., *Free Radic. Biol. Med*., 2001, vol. 30, p. 1191.
34. Battin, E.E., and Brumaghim, J.L., *Cell Biochem. Biophys*, 2009, vol. 55, p. 1.